as well as with OA progression in the knee joint cartilages of the mouse experimental model and human surgical specimens. In all systems, the expression was associated with factors related to endochondral ossification such as COL10A1, MMP3, 9, 13, VEGF, Indian hedgehog, PTH/PTHrP type I receptor, and Runx2. HIF2A enhanced promoter activities of these factors through specific binding to the respective hypoxia-responsive elements. The *Hif2a* heterozygous deficient (*Hif2a*+/-) mice exhibited slight growth retardation and notable resistance to OA development with decreased expressions of all factors above. The loss- and gain-of-function analyses in the cultures of ATDC5 cells and primary *Hif2a*+/- chondrocytes revealed that HIF2A was crucial for endochondral ossification, independently of the oxygen-dependent hydroxylation. Our further study on the upstream signal using the *HIF2A* promoter assay identified RELA, an NF-κB family member, as the most potent transactivator, and determined an NF-κB motif as the core responsive region by mutagenesis analysis. TNF-α and IL-1β, putative ligands for the NF-κB signal, increased the HIF2A expression in chondrogenic cells. In the mouse joint cartilage, the RELA expression was induced alongside the HIF2A expression during OA development. Finally, we have identified one common SNP (rs17039192; +18C/T) in 5'UTR of the human *HIF2A* gene in the ROAD population-based cohort, and the case-control association study using individuals over 50 years of age with (K/L grade $\geq$ 3; n=397) and without (K/L$\leq$1; n=437) knee OA showed a significant association of this SNP with knee OA (*P*=0.013, odds ratio=1.44). The *HIF2A* promoter containing the susceptibility allele (18C) showed higher transactivity than that containing 18T in chondrogenic cells with and without the RELA co-transfection; however, the difference was abrogated by the mutagenesis in the responsive NF-κB motif above, indicating the mediation of HIF2A transactivation by the NF-κB signal in the regulation of OA by the SNP.

Conclusions: The HIF2A/NF-κB signal controls extensive steps of endochondral ossification in OA development of mice and humans, so that this signal may represent a therapeutic target for OA.

019

A POSITIVE ROLE OF DBC1, A SIRT1 REPRESSOR, IN THE ARTHRITIC RESPONSE IN HUMAN CHONDROCYTES

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Purpose: Osteoarthritis (OA) is a multi-factorial disease featuring an imbalance between cartilage anabolism and catabolism. Chronic inflammatory stress contributes to enhanced matrix degradation and chondrocyte apoptosis in OA cartilage. We have shown that SirT1, a protein deacetylase known to prolong lifespan, is able to enhance both chondrocyte viability and cartilage specific gene expression. Here we evaluate the ability of DBC1 (Deleted in Breast Cancer 1), a repressor of SirT1, to regulate expression of the cartilage-specific genes collagen type 2 and aggrecan.

Method: Overexpression of DBC1 and Sirt1 in human osteoarthritic chondrocytes were performed by Amaxa electroporation. Antibodies against Sirt1 (Millipore), aggrecan, collagen 2, (Santa Cruz) and DBC1 (Abcam) were used in immunoblotting, immunohistochemistry and immunoprecipitation. PCR utilized aggrecan, collagen type 2, GAPDH primers and Taqman PCR mix (Applied Biosystem)

Results: Overexpression of SirT1 in human chondrocytes led to enhanced expression of aggrecan and collagen type 2. Further, SirT1 repress expression of many matrix metalloproteinases (MMPs). In contrast, tissue sections from OA patients revealed reduced protein levels of SirT1 and aggrecan and collagen type 2, with elevated levels of MMPs. We find that DBC1 is upregulated in OA cartilage compared to Normal cartilage. Moreover, when expressed in human chondrocytes, DBC1 represses these aggrecan and collagen type 2. Further, DBC1 is upregulated by TNFα in chondrocytes where cartilage genes are repressed and MMPs are elevated.

Conclusion: Our results indicate that the longevity protein SirT1 is a positive regulator of cartilage matrix in chondrocytes. Further, DBC1 displays characteristics of a pro-arthritic protein, due to its ability to block SirT1 enzymatic activity. That DBC1 is upregulated in OA and is induced by TNFα. Thus SirT1 has features of an anti-osteoarthritic enzyme, consistent with its ability to reduce the severity of age-associated diseases.

020

THE EXPRESSION OF THE ANTI-APOPTOTIC TRANSCRIPTION FACTOR NF-kappaB-P65 IS MARKEDLY DIMINISHED IN CHONDROCYTES OF MURINE OSTEOARTHRITIC CARTILAGE AND IN A SUBSET OF HUMAN OSTEOARTHRITIC CARTILAGE SAMPLES

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Purpose: Chondrocytes play a central role in cartilage pathology as seen in rheumatoid arthritis (RA) and osteoarthritis (OA) patients by a deranged synthesis of extracellular matrix (ECM) components and the enhanced release of ECM destructive metalloproteinases (MMPs). Nuclear factor-kappaB (NF-kappaB) is an important transcription factor in the regulation of MMPs, but is also regarded as a survival factor in cells. We studied the regulation of NF-kappaB-P65 in chondrocytes in rheumatoid arthritis, osteoarthritis, mouse models of arthritis and osteoarthritis and the functional consequences of decreased level of NFkappaB-P65.

Methods: We measured the level of NF-kappaB-P65 in freshly isolated chondrocytes of arthritic cartilage obtained from joint replacement surgery, cartilage of a spontaneous osteoarthritis mouse model (STR/ORT), cartilage of a streptococcal cell wall- and antigen-induced arthritis and cartilage from young (14 weeks) and old (>12 months) mice by Western blotting, immunohistochemistry or RT-qPCR. To study the functional consequences of decreased level of NF-kappaB-P65 in chondrocytes the murine H4 chondrocyte-cell line was stably transduced with a lentivirus expressing a short-hairpin RNA against NF-kappaB-P65 to reduce the NF-kappaB-P65 protein levels by a RNA interference approach. We selected several celllines that expressed different amounts of NF-kappaB-P65 protein. To study the biological consequences, conditioned medium of OA synovium was added to the murine chondrocyte cell line with the lowest NF-kappaB-P65 level.

Results: In all chondrocytes of RA patients high NF-kappaB-P65 levels were detected, by immunohistochemistry and Western blot, whereas in chondrocytes of a subset of OA cartilage samples levels were unexpected low (6 out of 12). In mouse models the level of NF-kappaB-P65 showed the same regulation. NF-kappaB-P65 levels in cartilage from murine arthritis models was increased up to 250% at day 2 after induction of streptococcal cell wall- or antigen-induced arthritis and at day 7 returned to the basal level of naive knee joints, whereas in STR/ORT mice levels were diminished more than 75% when joints became affected, as determined by immunohistochemistry. Levels of NF-kappaB-P65 in young and old mice were equal, but the older groups showed more variation, detected by immunohistochemistry. *In vitro*, we selected chondrocyte cell-lines with different levels of NF-kappaB-P65. By adding TNFalpha, cell death was only induced in the cells with low levels of NF-kappaB-P65, as detected by 7-AAD staining. A clear negative correlation between TNFalpha induced cell death and the levels of NF-kappaB-P65 in chondrocyte cell-lines was found. Adding conditioned medium of synovial explants from different OA patients to the murine chondrocyte cell-line with the lowest NF-kappaB-P65 level, resulted in more than 60% chondrocyte death in 3 of the 5 conditioned medium samples tested which could be prevented by preincubation of these media with soluble-TNFR1 (Enbrel). TNFalpha was detected using a Luminex assay in the same samples that caused cell death.

Conclusions: This study clearly demonstrated that lower levels of NF-kappaB-P65 makes chondrocytes more vulnerable for TNFalpha, a cytokine which can be produced during OA, and that this anti-apoptotic transcription factor is downregulated in chondrocytes in murine OA and in 50% of OA patients.

021

RESPONSIVENESS AND RELIABILITY OF MRI IN OSTEOARTHRITIS: ANALYTIC LITERATURE REVIEW

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Purpose: To summarize literature on the responsiveness and reliability of MRI-based measures of osteoarthritis (OA) structural change.

Methods: An online literature search was conducted of the OVID, EMBASE, CINAHL, PsychInfo and Cochrane databases of articles published up to the

time of the search, April 2009, with the search entries "MRI", and "osteoarthritis". The abstracts of the 1338 citations received with this search were then preliminarily screened for relevance by two reviewers. Of these, 243 were selected for data extraction for this analysis as well as a distinct analysis on validity. For this analysis we extracted data on responsiveness and reliability from both longitudinal and cross-sectional studies for all synovial joint tissues as it relates to MRI measurement in OA. Reliability was defined by inter- and intra-class correlation (ICC) and kappa statistics. Responsiveness was defined as standardized response mean (SRM)- ratio of mean of change over time divided by standard deviation of change. Random-effects pooling was used to summarize data from multiple studies.
Results: The reliability analysis (Table 1) included data from 89 manuscripts. The inter-reader and intra-reader ICC were all excellent (range 0.8-0.94).The inter-reader and intra-reader kappa values for quantitative, semi-quantitative and compositional measures were all moderate to excellent (range 0.52-0.88).
The responsiveness analysis (Table 2) included data from 42 manuscripts. The pooled SRM for quantitative measures of cartilage for medial tibiofemoral joint was -0.58 (95% CI -0.75 to -0.41). The pooled SRM for the semi-quantitative measurement of cartilage for the medial tibiofemoral joint was 0.55 (95% CI 0.47 to 0.64). For the quantitative analysis we have negative SRMs because the quantitative value goes down (indicating a loss of cartilage). For the semi-quantitative analysis, we have positive SRMs indicating a loss in cartilage because that is how the scale is defined.

Table 1. Results of random-effects pooling of *reproducibility* from MRI studies stratified by measure (quantitative, semi-quantitative) and tissue (cartilage, synovium, bone marrow lesion, meniscus, and ligament)

Inter-reader intra-class correlations (ICC)	Number of Estimates (Studies)	Mean Sample Size	Pooled ICC	95% CI
Quantitative				
Cartilage	10 (4)	196	0.90	0.86, 0.95
Meniscus	2 (1)	291	0.81	0.72, 0.89
Inter-reader kappa	**Number of Estimates (Studies)**	**Mean Sample Size**	**Pooled Kappa**	**95% CI**
Semi-Quantitative				
Cartilage	15 (4)	136	0.57	0.44, 0.71
Bone Marrow Lesion	2 (2)	237	0.88	0.79, 0.97
Meniscus	3 (3)	418	0.73	0.63, 0.84
Ligament	3 (3)	209	0.80	0.69, 0.90

CI: Confidence Interval.

Table 2. Results of random-effects pooling of standardized response mean from MRI studies stratified by measure (quantitative, semi-quantitative, and compositional) and tissue (cartilage, synovium, bone, bone marrow lesion, meniscus, and ligament)

Stratification	Number of Estimates (Studies)	Mean Sample Size	Pooled SRM	95% CI
Quantitative Cartilage				
Medial Femoral	54 (12)	118	-0.39	-0.48, -0.30
Medial Tibial	55 (17)	134	-0.33	-0.39, -0.26
Medial Tibio-Femoral	31 (12)	92	-0.58	-0.75, -0.41
Lateral Femoral	32 (8)	151	-0.19	-0.27, -0.11
Lateral Tibial	44 (14)	152	-0.44	-0.51, -0.36
Lateral Tibio-Femoral	14 (5)	110	-0.56	-0.92, -0.20
Patella	13 (9)	131	-0.60	-0.83, -0.37
Semi-Quantitative Cartilage				
Medial Tibial-Femoral	3 (3)	224	0.55	0.47, 0.64
Lateral Tibial-Femoral	3 (3)	224	0.37	0.18, 0.57
Patella	2 (2)	238	0.29	0.03, 0.56
Semi-Quantitative Other				
Synovium	3 (2)	68	0.52	0.28, 0.76
Osteophytes	4 (1)	150	0.36	0.28, 0.44
Bone Marrow Lesion	6 (2)	130	0.19	0.07, 0.30
Meniscus	2 (1)	264	0.27	0.14, 0.40

CI: Confidence Interval.

Conclusions: MRI has evolved substantially over the last decade and its strengths include its ability to visualize individual tissue pathologies, which can be measured reliably using both quantitative and semi-quantitative techniques. Using MRI it is possible to accurately and feasibly measure change in cartilage morphology over 12 months for knee OA. Studies also suggest that cartilage and synovium are responsive measures on semi-quantitative assessment.

022

MORPHOLOGIC DIFFERENCES BETWEEN POST-TRAUMATIC OA AND PRIMARY OA KNEES - DATA FROM THE OSTEOARTHRITIS INITIATIVE

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Purpose: A history of knee trauma is a risk factor for OA incidence, but it is unknown whether knees with radiographic OA (ROA) and a history of injury structurally differ from ROA knees without history of injury. We used MRI to investigate differences in total subchondral bone area (tAB) and subregional cartilage thickness (ThC) patterns between ROA knees with and without a history of injury from the Osteoarthritis Initiative.
Methods: Baseline MR images from the right knees of 891Osteoarthritis Initiative participants with ROA (public use data sets 0.E.1 [imaging] and 0.2.2 [clinical]; 309 had a history of trauma (i.e. answered yes to the question: Did you ever injure right knee badly enough to limit ability to walk for at least two days?), and 574 had not. Of knees with trauma history, 2% had *no* definite osteophytes (OPs), 28% had definite OPs and no JSN, 37% had isolated medial JSN, 21% had isolated lateral JSN, and 11% had bicompartimental JSN. In the non-trauma history knees, the numbers were 8%/38%/33%/14%/6% respectively; ThC results were stratified for these ROA categories. There was no difference in age (61 vs 63 years) and BMI (29.7 vs. 29.6) between ROA knees with and without trauma history, but more men than women had a history of knee injury (p=0.001). Thus, results for tAB were stratified by sex and results for ThC were adjusted for sex. Between group differences were compared using the Mann Whithney U-test (uni-variate) and general linear models (GLM, multi-variate). No correction for multiple testing was applied as this was an exploratory study.
Results: The tAB in the central aspect of the medial (p=0.001) and lateral (p=0.008) femoral condyles was significantly larger in male (but not in female) knees with trauma history than in those without. In knees with isolated medial JSN, ThC was significantly greater in the central lateral femur (cLF, p= 0.007) and tibia (LT, p=0.042) in those with trauma history vs. those without, after adjustment for sex. No other between groups difference was found on the cartilage plate level after adjustment. In knees with OPs and no JSN, the internal medial tibia (iMT) and internal lateral femur (icMF) displayed significantly greater ThC in knees with trauma than in those without (p=0.011/p=0.03, after adjusting for sex respectively); iMT displayed a similar difference also with isolated medial JSN (p=0.02) In knees with isolated medial (but not isolated lateral) JSN, all lateral subregions displayed greater ThC in those with trauma history than in those without; the differences being significant in the central (p=0.007), external (p=0.004), anterior (p=0.006) and posterior lateral tibia (p=0.023), and in the external central lateral femur (p=0.005) after adjusting for sex (Figure 1).

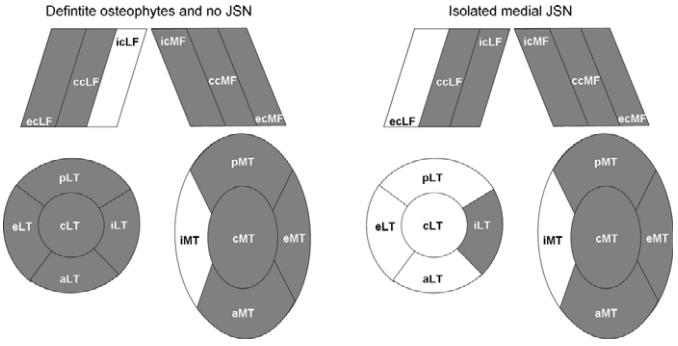

Figure 1. Differences in regional cartilage thickness pattern between those with a history of knee injury and those without. Dark grey subregions indicate no significant difference and white subregions indicate subregions where the cartilage was significantly thicker in those with a history of knee injury after adjusting for sex.

Conclusions: The results of this cross-sectional study suggest that there are discrete, but consistent morphologic differences between ROA knees with and without a history of knee injury. The larger femoral tAB in trauma knees may represent an adaptive response to the larger mechanical insults that lead to OA in injured knees. The pattern of thicker cartilage in iMT and icLF (in ROA knees without JSN) is interesting, as these cartilage subregions